Summary

Summary Chapter

In this thesis, it was shown that the assembly behavior of genetically engineered, supercharged polypeptides (SUPs) and their fusions with other proteins could be significantly expanded. This was achieved on the one hand by combining sequences with high net charge density with β-sheet forming motifs in an alternating fashion. On the other, SUPs were combined with oppositely charged proteins including spherical capsid structures to form cocrystals. Moreover, SUPs and their fusions were translated into diverse potential applications including surface functionalization to tune ice nucleation, as active components for the construction of a chemical tongue and for bioprotonic device fabrication.

In chapter 2, cationic and anionic SUPs were deposited on surfaces. It was demonstrated that the positively charged SUPs facilitate ice nucleation, while negatively charged SUPs suppress it. These experimental observations can be rationalized by considering the asymmetric polarization of interfacial water on positively and negatively charged surfaces. Thus, SUP materials hold great promise for the investigation of new generation anti-icing or cryopreservation solutions for biological materials in a medical context.

SUPs cannot only function on surfaces but their fusions can also play an important role as detection systems in solution. In chapter 3, we applied two different arrays consisting of either supercharged GFPs or fluorescent polyelectrolytes as a hypothesis-free chemical tongue that discriminates more than thirty whiskies based on their places of origin, brands, blend status or tastes. The underlying mechanism relies on the modulation of fluorescence intensity, i.e. quenching, of array elements triggered by analyte whiskies. Thereby, age, country of origin, blend status were successfully discriminated with an accuracy of 100% when both SUP fusions and conjugated polyelectrolytes were utilized in this chemical tongue set up.

The study of SUPs in Chapter 4 was also performed in aqueous solution. K72 and GFP-K72 underwent reversible self-assembly with both CCMV and aFT via electrostatic interactions to form so far unprecedented cocrystals. Electrolyte concentration that exceeded a critical point (~100 mM NaCl) screened the interactions and caused the structures to disassemble again. The same effect can most likely be achieved by adjusting the pH of the solution. All of the complexes assembled into macroscopic structures, demonstrating that additional functionalities can be embedded into the systems without preventing self-assembly. Here, this was demonstrated for GFP. Such structures resemble occlusion bodies found in nature and could find potential applications for maintaining the stability of delicate

biomolecules.

From the perspective of materials engineering, it is highly attractive to realize the performance improvement of a device in a stepwise manner. In chapter 5, we applied molecular design and genetic engineering to achieve a step-by-step increase of proton conduction. It needs to be emphasized that it is a very difficult task to improve the properties of protein-based bulk materials. The reason is that the properties of bulk materials are not only determined by a single molecule but strongly depends on the ensemble of molecules, their superstructures formation and their processing. For the evolution of proton conductivity, we started from completely unstructured anionic polypeptides. Then, we found that folded proteins with fixed charges yield higher proton conductivity compared to unstructured ones. Subsequently, we combined unstructured anionic SUP domains with β-sheet forming motifs yielding proton pathways bearing hierarchical structure formation. This architecture contributes to the final protonic conductivity and robust mechanics, respectively. The proton conducting abilities with a record value for biomacromolecules of 18.5 ± 5.5 mS/cm at RH = 90% was achieved. Moreover, the mechanical properties of the materials were improved during the design process eventually yielding free-standing membranes.